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Gametogenesis eliminates age-induced cellular damage and resets lifespan in yeast

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Abstract

Eukaryotic organisms age, yet detrimental age-associated traits are not passed on to progeny. How lifespan is reset from one generation to the next is not known. We show that in budding yeast resetting of lifespan occurs during gametogenesis. Gametes (spores) generated by aged cells show the same replicative potential as gametes generated by young cells. Age-associated damage is no longer detectable in mature gametes. Furthermore, transient induction of a transcription factor essential for later stages of gametogenesis extends the replicative lifespan of aged cells. Our results indicate that gamete formation brings about rejuvenation by eliminating age-induced cellular damage.

Most if not all eukaryotic organisms age, however, the age-induced changes are not transmitted to the progeny. How lifespan is reset from one generation to the next is not known. We wished to test the hypothesis that resetting of lifespan occurs during gametogenesis. In budding yeast, gamete formation (sporulation) requires meiosis and includes the generation of new membrane compartments, protein and organelle degradation, and synthesis of a resistant spore wall (1). To determine whether gamete formation causes rejuvenation, we asked whether spores derived from aged cells have reset their lifespan and are young or whether they inherit the progenitor's age and remain old. We isolated replicatively aged cells based on biotin labeling of mother cells (2) and induced them to sporulate in the same flask as young cells (Fig.S1). Upon sporulation, tetrads were dissected and the replicative lifespan (RLS) of each spore was measured. We found that the lifespans of the spores derived from young and aged cells were indistinguishable in two *Saccharomyces cerevisiae* strain backgrounds: W303, in which sporulation efficiency decreases with age ((3,4); Fig.1B) and A364a, in which sporulation efficiency remains high despite aging ((4);Fig.S2). In contrast, aged cells obtained by the same procedure but not induced to sporulate die rapidly (Fig.1A). The RLS of the four spores from a tetrad produced from young and aged cells is the same; no statistically significant differences are observed ((4); Fig.1C). This is in contrast to mitosis, where age is asymmetrically inherited between the mother cell and the bud, culminating in the production of a young daughter and an old mother cell (5). Thus, sporulation resets RLS.

We next asked how sporulation affects age dependent cellular changes such as increased levels of protein aggregation (6), aberrant nucleolar structures and increased levels of extra

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chromosomal ribosomal DNA circles (ERCs) (7,8). In budding yeast, protein aggregates associate with Hsp104 and form foci in replicatively aged cells, which can be visualized by Hsp104-eGFP ((6); Fig.S3A–D). During vegetative growth, Hsp104-eGFP foci are distributed asymmetrically between the mother and daughter cell; less than 10% of young cells displayed foci, but Hsp104-eGFP foci began to accumulate by generation 8 in mother cells and were present in 85% of aged cells ((4, 6, 9); Fig.S3D). Throughout sporulation most aged cells contained Hsp104-eGFP foci (mononucleates (95%), binucleates (90%) and tetranucleates (86%)), but the foci were essentially absent in mature tetrads (3%; Fig.2A–B), suggesting that age-associated protein aggregates are cleared during sporulation. The polarisome is required for the asymmetric distribution of Hsp104-eGFP foci during mitosis (9). Deleting the genes encoding the polarisome components Bud6 or Spa2 did not interfere with aggregate elimination during sporulation (Fig.S3E–F). Proteasome function also appeared dispensable for aggregate clearance. Treatment of cells with the proteasome inhibitor MG132 after the second division neither prevented sporulation nor the clearance of Hsp104 aggregates ((10); Fig.S3G–H). In contrast, treatment of cells with the autophagy inhibitor chloroquine prevented sporulation and aggregates persisted ((11); Fig.S3G–H), suggesting that an autophagy-dependent process and/or spore formation are required for aggregate clearance.

Aged cells are also defective in rDNA metabolism, displaying fragmented nucleoli and forming extra chromosomal rDNA circles (ERCs) (7,8). ERCs decreased considerably during sporulation in aged cells, reaching levels similar to young cells (Fig.2C, Fig.S4). In aged cells, the rDNA structure also underwent dramatic changes as judged by the localization of Fob1-GFP, a nucleolar protein that binds to the rDNA (12). In 60% of aged cells, the Fob1-GFP appeared enlarged and discontinuous, likely reflecting rDNA condensation defects and nucleolar fragmentation, respectively. After aged cells sporulate, more than 90% of the tetrads contained a single Fob1-GFP focus per spore, and displayed a morphology indistinguishable from that of young cells (Fig.2D). Together, our results demonstrate that gamete formation eliminates age-induced protein aggregation and nucleolar aberrations.

To determine which aspects of gametogenesis are necessary for RLS resetting, we deleted two transcription factors that trigger different stages of sporulation and asked if RLS was reset. Such studies are possible, because in budding yeast, sporulating cells can resume vegetative growth (return to growth; (4)) provided that the sporulation-inducing cue, nutrient deprivation, is withdrawn. We first analyzed the RLS of young (1.4±0.6 generations) and aged (16.2±4 generations) cells from a strain that lacks Ime1. Without Ime1, yeast cells are unable to initiate sporulation but still sense nutrient deprivation (13). To ensure that only cells that responded to the sporulation-inducing cues were included in the analysis, we used a *pIME1:mCherry* reporter construct (Fig.S5A) (14). Aged *ime1Δ* cells lost viability rapidly, with a median lifespan of 4 generations (Fig.3A). Similar results were obtained with wild-type cells that had responded to sporulation cues as judged by *pIME1:mCherry* expression but had not yet entered the sporulation program (Fig.S5B) Thus, the initiation of sporulation driven by *IME1* is required to reset RLS. Furthermore, nutrient deprivation and other sporulation signals are insufficient to promote RLS resetting.

In the absence of the transcription factor Ndt80, yeast cells complete pre-meiotic DNA replication, initiate recombination and arrest in pachytene (15). We used Zip1-GFP to identify the *ndt80Δ* cells arrested in pachytene (Fig.S5C); (16) and found that young cells (1.3±0.6 generations) resumed vegetative growth and divided an average of 21.6±7.9 times (median RLS=21). In contrast, almost 50% of the aged cells (16.8±4.4 generations) lost viability within the first mitotic division. The remaining cells underwent significantly fewer divisions compared to young cells (3.4±4.9 generations; Fig.3B). We conclude that *NDT80-*

induced processes are necessary for RLS resetting, and that the events prior to *NDT80* function such as pre-meiotic DNA replication and recombination are insufficient to promote rejuvenation.

Progression through sporulation up to pachytene is not sufficient for resetting of RLS. Thus, later stages of sporulation, the meiotic nuclear divisions and/or spore formation must be required. To determine whether both meiotic divisions are necessary for RLS resetting, we deleted *SPO12. spo12Δ* cells undergo a single nuclear division and form two diploid spores (17). We found that young (1.3±0.6 generations) and aged (14±1.8 generations) *spo12Δ* cells had indistinguishable RLS, suggesting that two consecutive meiotic divisions are not a prerequisite for rejuvenation (Fig.3C). The lifespan of spores within individual two-spored asci is very similar (Fig.S6) (p [young]= 0.15, p [aged]= 0.29, n=30, Wilcoxon signed-rank test). To test if resetting of RLS can occur in the absence of any nuclear divisions, we inactivated the polo-like kinase Cdc5 during sporulation (*cdc5-mn*; (18)). Cells lacking Cdc5 do not undergo any meiotic divisions and form single spores. Like *spo12Δ* spores, *cdc5-mn* spores obtained from aged cells regained their replicative potential (Fig.3D). We conclude that the meiotic divisions *per se* are dispensable for RLS resetting and note that our findings exclude a model where halving of the genome or diluting aging factors brings about the resetting of RLS (4).

NDT80-regulated genes that mediate spore formation could be required for rejuvenation. As *NDT80* expression is sufficient to induce the expression of mid and late sporulation genes in vegetative cells (15, 19) we determined whether Ndt80 could extend the lifespan of vegetative cells. We expressed *NDT80* from the *GAL1-10* promoter (*GAL-NDT80*), whose expression can be regulated by a Gal4-estrogen receptor fusion (Gal4.ER) ((20, 21); Fig.S7A–B). Expression of *NDT80* significantly extended the lifespan of mitotic cells (Fig.S7C–D; P-value <0.0001, Z-score=4, Mann-Whitney test). To test whether induction of *NDT80* in replicatively aged cells also extends lifespan, we transiently induced Ndt80 with β-estradiol in young and aged cells and followed their RLS in the absence of β-estradiol. Aged cells that transiently expressed *NDT80* lived significantly longer than aged cells treated in the same manner but lacking the *GAL-NDT80* fusion (Fig.4A, P-value < 0.0001, Z-score=8.23, Mann-Whitney test). Transient expression of *NDT80* even led to an extension of lifespan in young cells (Fig.4A, P-value < 0.0001, Z-score=4.85, Mann-Whitney test). Similar results were obtained in experiments comparing cell divisions in liquid culture, excluding the possibility that transient expression of *NDT80* extends life span because it causes cells to become more resistant to the micromanipulations involved in the pedigree analysis (Fig.4B–C). Thus, a transient induction of *NDT80* is sufficient to extend the lifespan of replicatively aged cells.

To address how transient induction of Ndt80 extends RLS, we monitored age-dependent cellular changes after *NDT80* induction. Neither ERCs nor Hsp104-eGFP aggregates were reduced after *NDT80* induction, although it is possible that they were reduced at later time points (Fig.S8A–B). Furthermore, *NDT80* expression extended lifespan in the absence of the autophagy gene *ATG1* (Fig.S8C). Together these findings suggest that lifespan extension can occur in the absence of ERC and Hsp104-aggregate elimination. While ERCs and Hsp104-eGFP aggregates were not affected by transient *NTD80* expression, nucleolar morphology was. The percentage of aged cells with enlarged nucleolar morphology decreased after *NDT80* induction (Fig.4D–E, Fig.S8D). Thus, transient induction of *NDT80* causes a change in nucleolar/rDNA structure reverting it to a state that resembles the morphology in young cells.

We do not yet know whether *NDT80*- and sporulation-induced RLS resetting use the same mechanism(s). The findings that *NDT80* is necessary for lifespan extension during

sporulation and sufficient for lifespan extension during vegetative growth and that nucleolar morphology is altered under both circumstances, suggest that at least some processes are shared. Irrespective of the relationship between *NDT80*- and sporulation-induced RLS resetting, we note that resetting of RLS provides an opportunity to dissect the molecular causes of aging. For instance, elimination of Hsp104 aggregates and ERCs seem unlikely to be required for *NDT80*-dependent lifespan extension, but changes in nucleolar function and/or structure may be important. Intriguingly, rDNA instability and not ERCs *per se* appear to cause aging in yeast (22) and budding yeast cells eliminate most of the nucleolar material during spore packaging (23).

It will be interesting to investigate whether our findings extend to other species. In *C. elegans*, a number of longevity mutants exhibit a soma-to-germline transformation that contributes to their enhanced survival (24). In mice, reintroduction of telomerase rescues the age-related phenotypes of telomerase-deficient mice (25), suggesting that age-dependent cellular damage can be repaired. Our studies suggest that a transient induction of the gametogenesis program in somatic cells removes age-dependent cellular damage and extends lifespan. Determining how gametogenesis causes the resetting of lifespan will provide insights into the mechanisms of aging and could facilitate the development of strategies for longevity.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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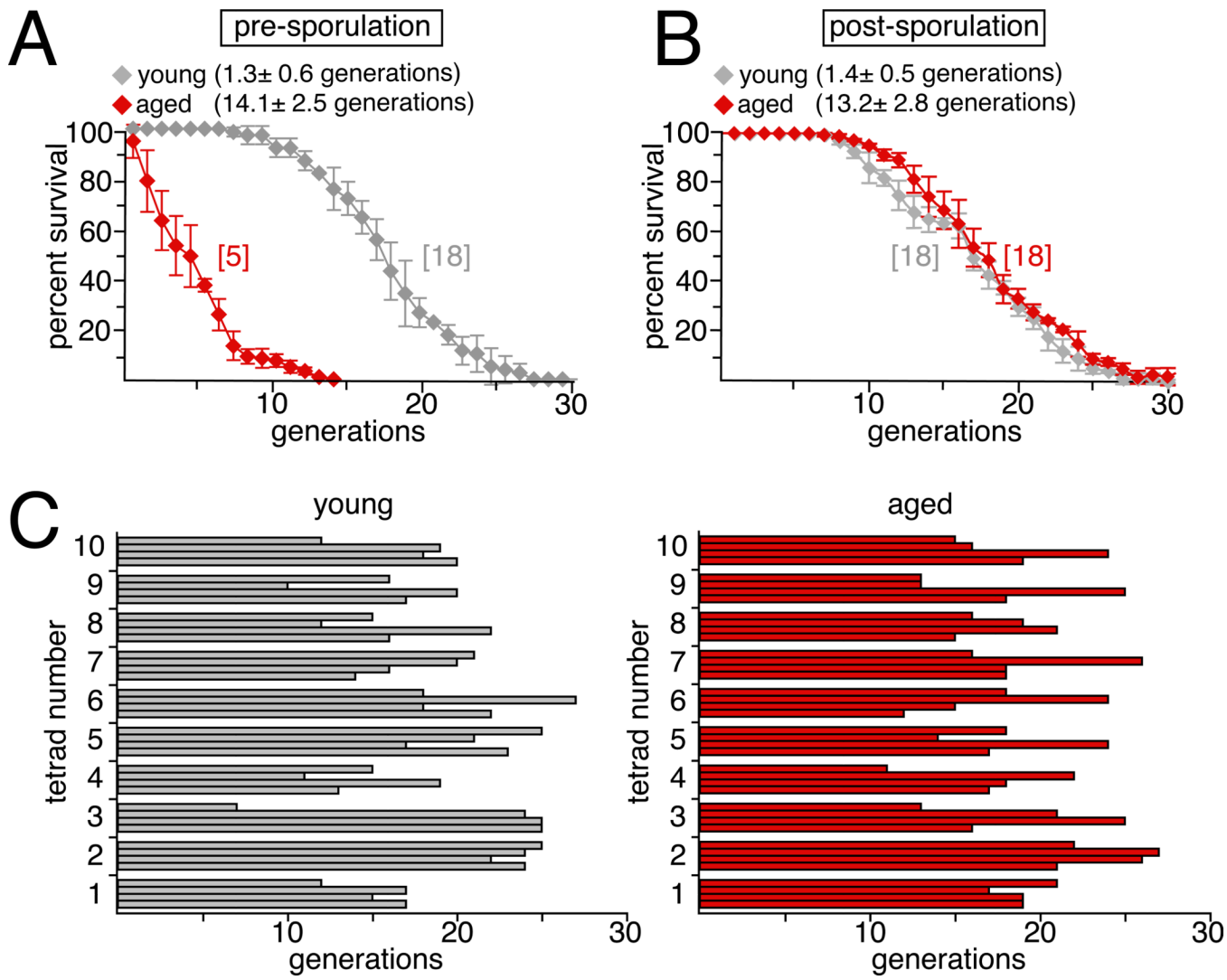

Figure 1. Gametogenesis resets RLS

The average number of cell divisions of the starting cell population is indicated in the graph legend. The median lifespan is written next to each curve. Error bars denote S.D.

A) RLS of young and aged wild-type A702 cells directly after sorting.

B) Post-sporulation RLS of spores from young and aged A702 cells.

C) Age distribution of spores from A702 in individual tetrads from young and aged progenitors, n=10. The lifespan of spores from each tetrad is compared to the mean lifespan of young spores to obtain a P-value. The average P-value from 10 tetrads is 0.303 for young and 0.642 for aged spores (t-test), indicating no statistically significant difference in replicative age among spores from a given tetrad.

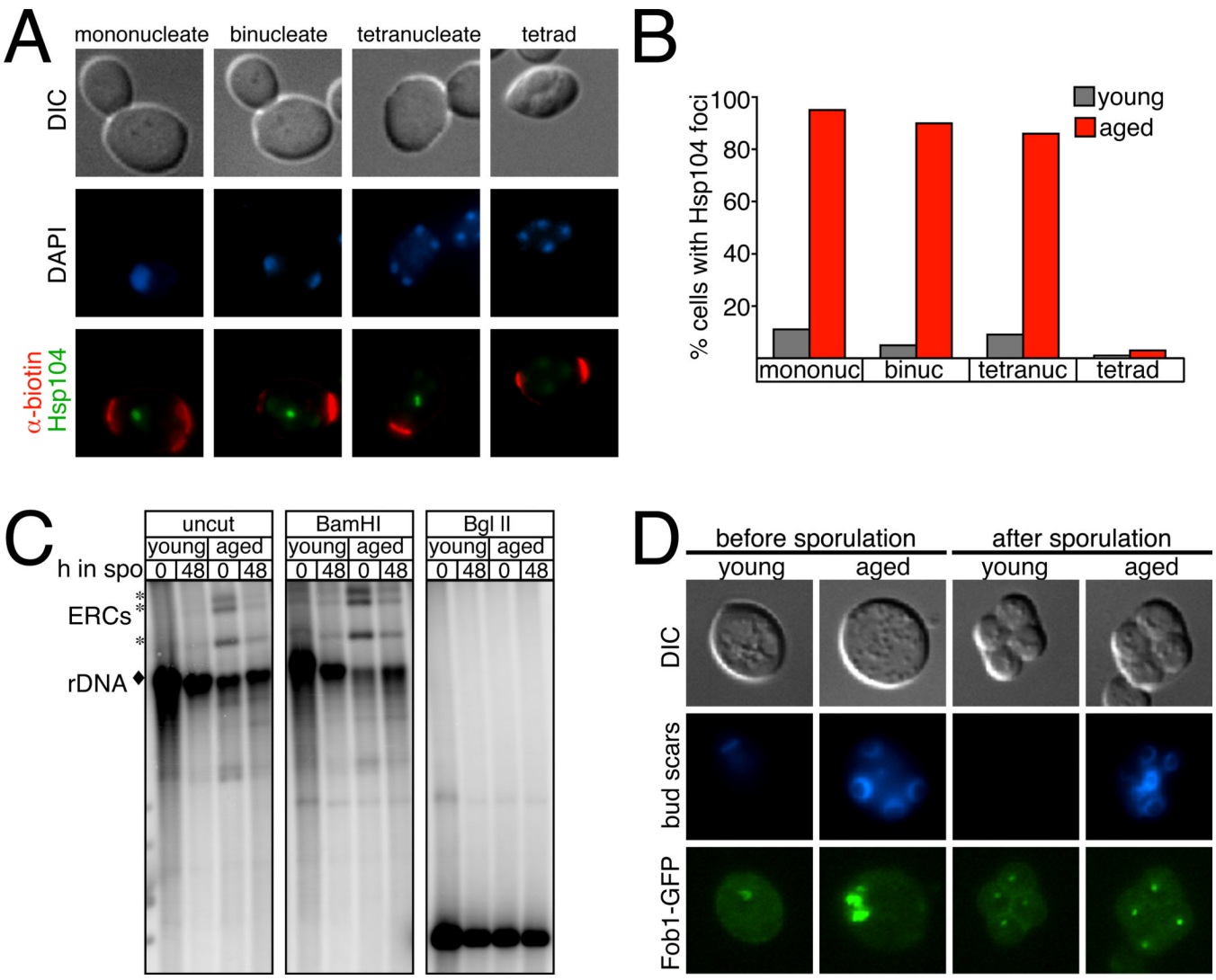

Figure 2. Sporulation eliminates age-induced cellular damage

A) Analysis of Hsp104-eGFP aggregates in aged sporulating A25825 cells.

B) Quantification of Hsp104-eGFP foci in young and aged A25825 cells prior to meiosisI (mononuc), after meiosisI (binuc), after meiosisII (tetranuc) and in tetrads.

C) rDNA and ERCs in young and aged A26370 cells.

D) Nucleolar morphology in young and aged A26271 cells.

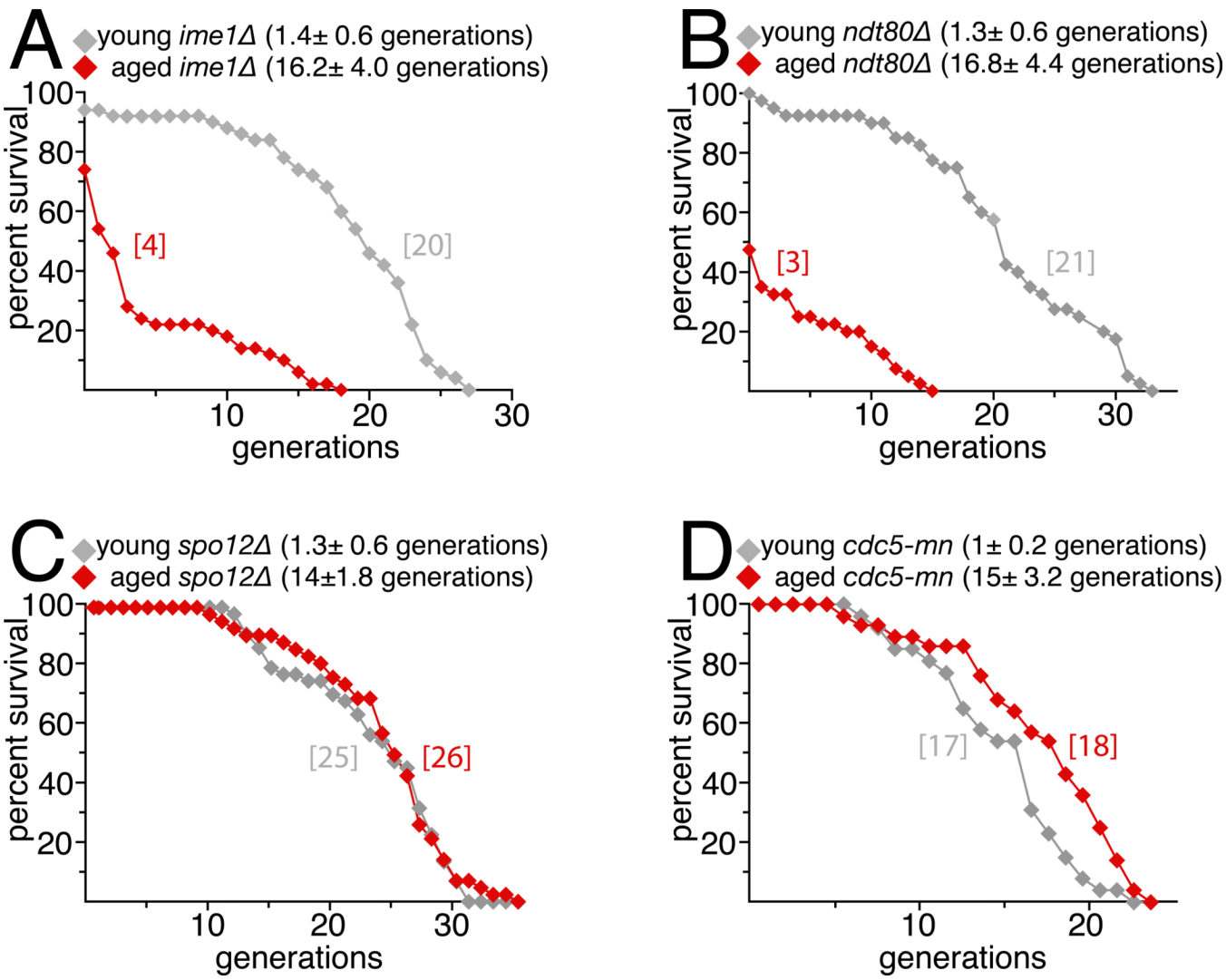

Figure 3. *IME1* and *NDT80* but not the meiotic nuclear divisions are required for lifespan resetting

A) RLS of young and aged A23998 (*ime1*Δ) cells.

B) RLS of young and aged A24074 (*ndt80*Δ) cells. The median lifespan of the aged cells is 0. Therefore, the average is shown.

C) The lifespans of young and aged A27377 (*spo12*Δ) cells.

D) The lifespans of young and aged A24142 (*cdc5-mn*) spores.

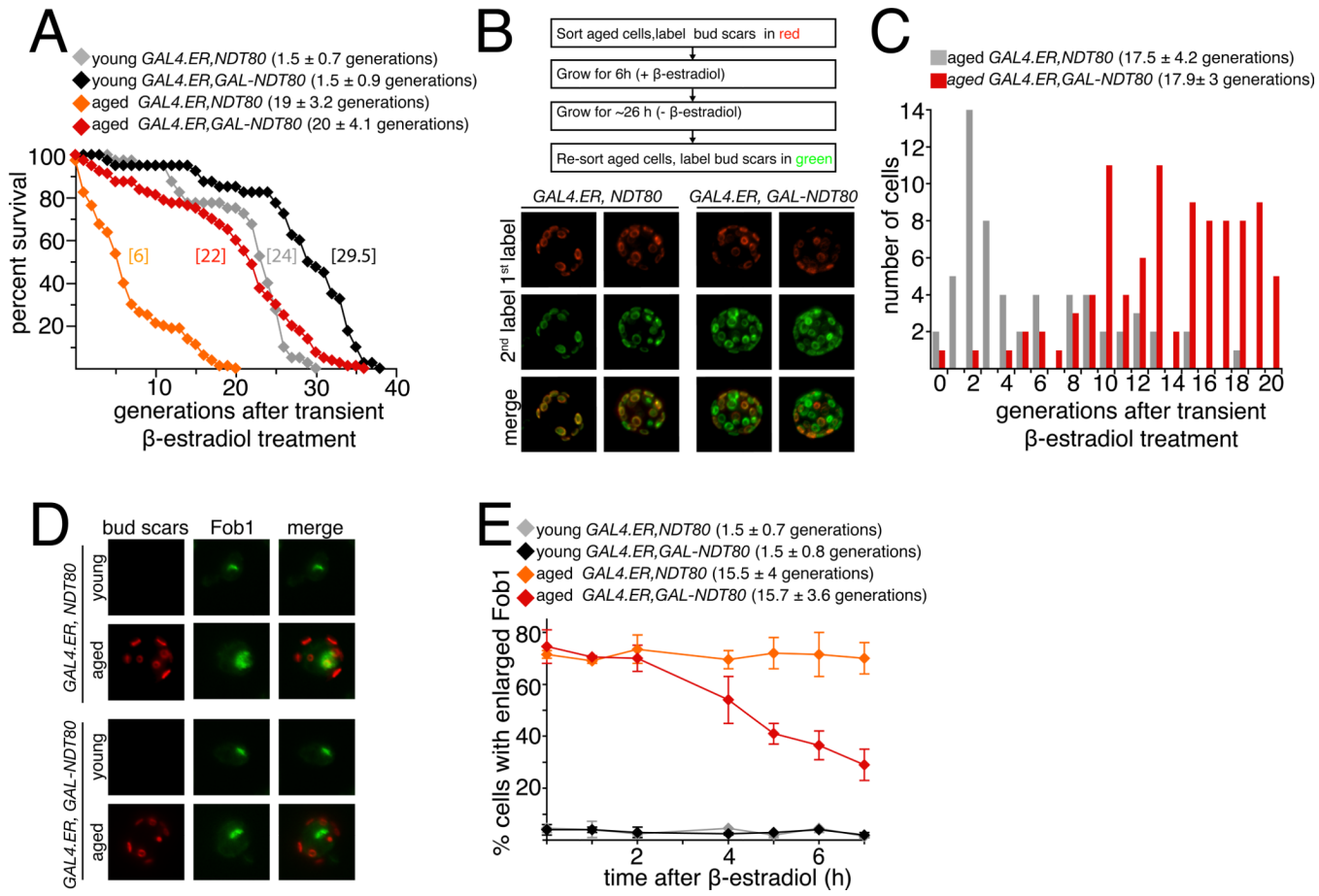

Figure 4. Transient *NDT80* expression extends the lifespan of vegetatively growing aged cells

A) Lifespan of young and aged cells from A25823 (*GAL4.ER*, *NDT80*) and A25824 (*GAL4.ER*, GAL-*NDT80*).

B) Top; description of the experiment, bottom; aged cells from A27507 (*GAL4.ER*, *NDT80*) and A27484 (*GAL4.ER*, GAL-*NDT80*) labeled before and after β-estradiol.

C) The number of cell divisions after β-estradiol treatment was calculated by the difference between the green- and red-labeled bud scars. The distribution of n=60 cells is shown for A27507 (*GAL4.ER*, *NDT80*) and n=100 cells for A27484 (*GAL4.ER*, GAL-*NDT80*).

D) Fob1-GFP in young and aged cells from strains A27507 (*GAL4.ER*, *NDT80*) and A27484 (*GAL4.ER*, GAL-*NDT80*) after 6th β-estradiol treatment.

E) Percentage of cells with enlarged Fob1-GFP from A27507 (*GAL4.ER*, *NDT80*) and A27484 (*GAL4.ER*, GAL-*NDT80*) following β-estradiol treatment. The average of two independent experiments is shown. 100 – 200 cells were counted for each time point; error bars display the range.